sciendo

DOI: 10.2478/fv-2021-0034

FOLIA VETERINARIA, 65, 4: 20—30, 2021

A FIELD STUDY EVALUATING HUMORAL IMMUNITY IN CALVES VACCINATED WITH MULTIVALENT BOVINE RESPIRATORY PATHOGEN VACCINES

Berge, A. C.[1], Jozan, T.[2], Levesque, C.[3], Vertenten, G.[4]

[1]Berge Veterinary Consulting BV, Oulstbergstraat 24, 1570 Vollezele
Belgium
[2]MSD Santé Animale, 7 Rue Olivier de Serres, Beaucouzé
[3]LABOCEA, 10 bis Rue Claude Bourgelat, 35133 Javené
France
[4]MSD Animal Health, W. de Körverstraat 35, Boxmeer
The Netherlands

cat@bergevetconsulting.com

ABSTRACT

Bovine Respiratory Syncytial Virus (BRSV), Bovine Parainfluenza 3 (BPI3) and *Mannheimia haemolytica* (*Mh*) are major respiratory pathogens in the bovine respiratory disease complex. It is important to optimize passive and active immunity to these pathogens early in life to reduce clinical and subclinical productivity losses. The administration of inactivated, adjuvanted and multivalent vaccines, such as Bovilis® Bovipast RSP (Bovipast), and Bovalto® Respi 3 (Bovalto) to calves, may enhance cellular and humoral immunity against BRSV, BPI3 and *Mh*. A field trial evaluated the immune responses to these three agents in the first year of life in 12 Bovipast and 13 Bovalto vaccinated calves, and 5 negative control calves. Calves were vaccinated starting at 2 weeks of age and revaccinated 4 weeks later (primo vaccination). A booster vaccination was given at approximately 10 months of age. Serum samples were taken at time intervals up to 6 months after primo vaccination and up to 1 month after the booster vaccination. BRSV serum titres were evaluated using a serum neutralisation assay (SN), and BRSV, BPI3 and *Mh* titres were evaluated using a commercial enzyme linked immunosorbent assay (ELISA) test. Serum antibodies after primo and booster vaccinations in the individual calves were evaluated by calculating the areas under the curve (AUC) of the Log2 transformed BRSV SN titres and the optic density measures of the ELISA tests for BRSV, BPI3 and *Mh*. Multivariate general linear models were used to evaluate the influence of the vaccination on the AUC of the serum measures within 6 months after the primo vaccination. Similarly, models evaluated the AUC of the serum measures after the booster vaccination. The Bovipast vaccinated calves had significantly higher SN and ELISA titres AUC following the primo vaccination and booster vaccinations compared to the negative control calves and the Bovalto vaccinated calves. The Bovalto vaccinated calves did not have a significantly different BRSV SN and ELISA titres AUC response after the primo or booster vaccinations compared to the negative control calves. The serum antibody responses to BPI3 and *Mh* in the vaccinated calves were less pronounced than the Bovipast BRSV antibody response. Bovipast and Boval-

to vaccinated calves mounted a significantly higher AUC ELISA OD for both BPI3 and *Mh* and the highest AUC was measured in the Bovipast vaccinated calves. This study indicated that early vaccinations of calves with multivalent adjuvanted inactivated BRD vaccines, such as Bovilis® Bovipast RSP can elicit a humoral response with a cellular-mediated memory effect as indicated by the booster vaccination.

Key words: Bovine Respiratory Disease; BPI3; BRSV; comparison; field study; *Mannheimia haemolytica*; neutralization; respiratory; vaccination

INTRODUCTION

Bovine respiratory disease (BRD) in calves is a complex and multifactorial disease, causing huge financial losses to the dairy and beef industry worldwide in veterinary treatment costs and reduced performance [3, 4, 13]. Bovine Respiratory Syncytial Virus (BRSV), Bovine Parainfluenza 3 virus (BPI3), bovine herpesvirus type 1 (BHV-1), bovine coronavirus (BCoV), *Mannheimia haemolytica* (*Mh*) and *Mycoplasma bovis* are considered as major or primary agents able to initiate BRD pathology. Other agents like *Pasteurella multocida*, *Histophilus somni* are generally considered as secondary or opportunist pathogens. With increasing threats of antimicrobial resistance due to antimicrobial prophylactic, metaphylactic and therapeutic BRD treatments, multivalent BRD vaccines are of increasing importance.

The bovine respiratory syncytial virus (BRSV) is a major pathogen within the BRD complex and cattle populations around the world are infected. Seroprevalences in infected herds can reach nearly the entire herd and many countries report that most herds are seropositive. BRSV infection predispose calves to secondary bacterial infection, resulting in the BRD complex [18].

BRD is mostly affecting young calves from the first days of life and BRSV-associated respiratory disease is most pronounced in calves less than 6 months of age. Therefore, it is important to optimize passive and active immunity to these pathogens early in life to reduce clinical and subclinical productivity losses. Pregnant cows could be vaccinated to transfer specific immunity by colostrum and calves may be vaccinated early in life to develop their own immunity quickly. However, some vaccines may be adversely affected by interference from maternal antibodies or unfavourable nutritional conditions [2]. As BRD may affect young calves after the decline of the maternal antibodies, vaccination against BRD pathogens should target calves in the first weeks of life, when maternal antibody levels are still reasonably high.

It is difficult to draw general conclusions about the immunity and protection induced by BRD vaccines. Differences in strains and adjuvant composition, or other differences in formulation, such as antigenic mass, the inactivation process, may be responsible for induction of disparate immune responses by 2 apparently similarly adjuvanted vaccines [9].

The multivalent, adjuvanted and inactivated respiratory disease vaccines Bovilis® Bovipast RSP (Bovilis Bovigrip: product name in France/ Bovigrip® RSP plus: product name in Germany) (Bovipast) from MSD Animal Health and Bovalto® Respi 3 (Bovalto) from Boehringer Ingelheim can be administrated to calves from two weeks of age and these contain inactivated BRSV, BPI3 and *Mh* strains. For Bovipast it has been demonstrated that protection against BRSV, BPI3 and *Mh* infection is provided even in the presence of maternal antibodies. The Bovalto vaccination efficacy has only been proven in seronegative animals, and the efficacy of the vaccination has not been demonstrated in the presence of maternal antibodies. Consequently, the level of antibody response after Bovalto administration may be reduced by maternal antibodies. It is difficult to find and keep calves seronegative for these respiratory pathogens in commercial farms since most dairy cows have been vaccinated or exposed to the viruses and transmit colostral antibodies to the calf. The BRSV seroprevalence in infected herds can reach nearly the entire herd and many countries have reported the prevalence of infected herds of 80 % or higher. Also, a BPI3 seroprevalence above 50 % is commonly reported in European countries [18]. Serum antibody response may be used to evaluate vaccination response and clinical risk of a respiratory challenge with BRSV has been shown to be correlated with neutralising antibody titres [25]. Virus neutralisation is a technique that makes it possible to objectively evaluate and compare the neutralisation capacity of the serum from vaccinated animals.

The objective of this field study was to evaluate the seroneutralising antibody responses against BRSV and specific humoral (IgG) response to BRSV, BPI3 and *Mh* in

young calves vaccinated with either Bovipast or Bovalto and non-vaccinated calves between 15 to 30 days of age, and after revaccination 9 to 11 months later.

MATERIALS AND METHODS

This study was performed on a dairy farm officially free from bovine viral diarrhoea (BVD) and infectious bovine rhinotracheitis (IBR) in Maine-et-Loire, France. The farm had no previous history of BRD in calves and no BRD vaccinations had been performed. Thirty calves born at the farm during the fall season of 2016 were included. All calves received at least three litres of colostrum during the first 6 hours of life according to farm protocols and were healthy at the time of the enrolment. All calves were raised under the same conditions as regards management, housing and feeding throughout the study. The calves were raised in individual igloo type hutches separated by at least a 40 cm space, thus not allowing nose to nose contact until after weaning at 2.5—3 months of age. After the first colostrum feed, the calves were fed farm whole fresh milk. After weaning between two to three months the calves were placed in group housings of 4—5 calves per group for a few weeks. Thereafter the calves were mixed in larger groups of approximately 20 calves in semi-enclosed buildings providing protection from rain and wind. There was no introduction of calves from other farms during the study period.

Healthy calves were randomly allocated to either vaccine groups Bovipast or Bovalto, or non-vaccinated Control group during each vaccination visit by the local practitioner who performed the vaccinations. Control calves were included in the first 5 vaccination visits to evaluate farm background levels of exposure to the respiratory agents. The calves were sequentially allocated to treatment and were group housed mixed together after the individual hutch period. The Bovipast group consisted of 12 calves, the Bovalto group of 13 calves and the control group of 5 calves. Both vaccines are composed of three inactivated pathogens. The Bovipast vaccine contains the BRSV strain EV908, PI3 strain SF-4 Reisinger and *M. haemolytica* A1 strain M4/1-IRP. The Bovipast vaccine used were in 50 ml bottles and the vaccine dose was 5 ml. The Bovalto vaccine contains the BRSV strain BIO-24, PI3 strain BIO-23 and *M. haemolytica* A1 strain DSM 5283. The Bovalto vaccines used were in 10 ml bottles and the vaccine dose was 2 ml. The study calves were in contact with non-study calves in the group housing pens.

The calves in the two vaccine groups were primo vaccinated at an age of 14—28 days (T0) and a second time 1 month later (T0+1m) and booster vaccinated at 9—11 months of age (T10). Blood samples were taken at T0, and thereafter every 2 weeks until 3 months after T0 (T0+0.5m, T0+1m, T0+1.5m, T0+2m, T0+2.5m, T0+3m). Thereafter, monthly blood samples were taken between 4 and 6 months after the first vaccination (T0+4m, T0+5m, T0+6m). Serum samples were taken at the booster vaccination (T10), and thereafter 2 weeks (T10+0.5m) and 1 month (T10+1m) after T10.

The serum was collected and transported cooled to the laboratory. All samples were analysed with ELISA kit BioX K 369 MULTISCREEN for serodiagnosis of BRSV, BPI3, and *Mh* (BioX Diagnostics SA, Rochefort, Belgium). The tests were 96-well microtitration plates sensitized with monoclonal antibodies specific to BPI3 and BRSV and purified lipopolysaccharide (LPS) of *Mh*. The seroneutralisation assay previously described was adapted for BRSV seroneutralising antibody detection [5]. Heat inactivated serum samples were two-fold diluted in minimum essential medium (1:2 to 1:4096) in 96-well plates. One hundred TCID 50.50 μl^{-1} of the 220/69 virus strain (from Belgium) suspension were added to each well. After homogenization, plates were incubated one hour at 35 °C ± 1 °C and 5 % CO_2. A bovine foetal kidney cell suspension containing 100 000 cells.ml^{-1} was added in each well. After homogenization, plates were incubated 4 to 5 days at 35 °C ± 1 °C and 5 % CO_2. Cell culture control, positive and negative control sera were added in each assay. Virus strain suspension used in the assay was also titrated. Conformity of all these parameters was checked for validation of the assay. The end-point titre was determined as the inverse of the highest dilution of serum that inhibited viral cytopathogenic effects. Seroconversion was defined as a four-fold increase in BRSV titres. The optic density (OD) measures of the ELISA tests and Log2 of SN titres were used as outcomes.

The results were entered into a spreadsheet by the study investigator. The data were verified and subsequently analysed using the statistical software SAS 9.4. The number and % of calves in the respective groups that had SN titres ≥ 16 (4Log2) were evaluated. Univariate analysis was performed of the outcomes and the calves respective AUC

values to evaluate their distributions for further statistical modelling [15]. To determine the effect of the level and duration of immunity, the area under the curve (AUC) was calculated for the period T0+1.0m to T0+6m (AUC-primo), and for the period T10m to T10+1m (AUC-booster) using Log2 transformed BRSV serum titres and ELISA OD measures of the BPI3 and *Mh*. General linear models (GLM) were used for the three agents, to evaluate the influence of the vaccination on the AUC of the serum measures within 6 months after the primo vaccination (AUC-primo) with age at vaccination (T0-age) and AUC of serum measures at the time of the primo vaccination (AUC-base) as covariates. Similarly, general linear models evaluated the influence of the vaccination on the AUC of the serum measures after the booster vaccination (AUC-booster) with the serum titres at the time of the booster vaccination (T-booster) as covariates.

RESULTS

All of the enrolled calves completed the first 6-month follow-up period of the primo vaccination. Two calves in the Bovalto group were lost to follow up in the booster vaccination. From each calf, ten serum samples were obtained following the primo vaccination and 3 serum samples were obtained following the booster vaccination. No BRD associated diseases were recorded during the study. The mean serum titres per calf group are described in Table 1 and Figures 1 through 4.

The number and percentage of calves with serum SN titres for BRSV ≥ 16 is described in Table 2. Over the complete study period, after the primo vaccination, 83 % of Bovilis Bovipast RSP® vaccinated calves, 31 % of Bovalto 3® vaccinated calves and 20 % of control calves had seroneutralisation titres ≥ 1 : 16 (4Log2) at one or several sampling times. The Bovalto vaccinated calves and the control calves

Table 1. Mean Log 2 serum neutralisations (SN) titres for Bovine Respiratory Syncytial Virus (BRSV) and ELISA optic density (OD) measures for BRSV, Bovine Parainfluenza 3 (BPI3) and *Mannheimia haemolytica* (*Mh*)

Agent	Group	Months after T0. Mean Log2 SN titres for BRSV												
		T0	T0+0.5	T0+1	T0+1.5	T0+2	T0+2.5	T0+3	T0+4	T0+5	T0+6	T10	T10+0.5	T10+1
BRSV	Bovipast	1.7	1.9	1.3	2.9	2.6	2.8	2.7	2.6	2.5	1.9	1.3	5.3	4.2
BRSV	Bovalto	2.8	2.4	1.9	1.9	1.8	1.5	1.3	0.8	0.7	0.7	0.5	1.2	1.2
BRSV	Control	3.4	2.0	1.4	1.8	0.8	0.6	0.2	0.0	0.0	0.4	0.6	0.8	0.2
Agent	Group	Months after T0. Mean ELISA OD for BRSV												
		T0	T0+0.5	T0+1	T0+1.5	T0+2	T0+2.5	T0+3	T0+4	T0+5	T0+6	T10	T10+0.5	T10+1
BRSV	Bovipast	45.6	35.7	32.6	87.9	78.2	76.0	75.9	64.2	50.2	38.4	45.9	106.1	109.1
BRSV	Bovalto	50.6	41.3	42.3	60.2	45.4	37.9	40.0	37.2	27.6	20.5	34.6	86.8	85.3
BRSV	Control	64.1	48.1	36.3	33.2	26.8	16.1	22.7	11.3	18.3	12.0	17.8	16.1	12.9
Agent	Group	Months after T0. Mean ELISA OD for BPI3												
		T0	T0+0.5	T0+1	T0+1.5	T0+2	T0+2.5	T0+3	T0+4	T0+5	T0+6	T10	T10+0.5	T10+1
BPI3	Bovipast	56.6	60.9	77.0	131.0	123.3	115.4	108.7	89.1	73.3	62.2	100.9	130.8	123.3
BPI3	Bovalto	72.5	71.4	73.1	115.8	102.0	82.4	78.7	73.3	68.4	56.4	99.7	116.8	118.3
BPI3	Control	69.9	58.2	50.0	34.0	34.0	23.4	42.1	70.2	60.3	40.2	105.4	115.7	106.7
Agent	Group	Months after T0. Mean ELISA OD for *Mh*												
		T0	T0+0.5	T0+1	T0+1.5	T0+2	T0+2.5	T0+3	T0+4	T0+5	T0+6	T10	T10+0.5	T10+1
MH	Bovipast	59.6	47.0	41.1	42.5	41.3	46.0	54.2	138.3	183.2	210.1	80.8	99.1	87.3
MH	Bovalto	74.1	67.9	42.3	34.8	31.7	44.1	48.5	120.2	162.2	174.3	78.3	91.8	79.4
MH	Control	57.1	48.5	37.5	33.0	25.2	27.1	24.1	42.5	117.4	114.1	64.9	69.7	60.8

Table 2. Number of calves tested and percentage of calves that had serum neutralisations (SN) titres) ≥ 16 to Bovine Respiratory Syncytial Virus (BRSV)

Group	N	T0	T0+0.5	T0+1	T0+1.5	T0+2	T0+2.5	T0+3	T0+4	T0+5	T0+6	N	T10	T10+0.5	T10+1
Bovipast	12	8%	17%	0%	50%	33%	33%	50%	42%	25%	17%	12	0%	83%	66%
Bovalto	13	31%	31%	23%	23%	15%	0%	0%	0%	0%	0%	11	0%	0%	0%
Control	5	60%	20%	20%	20%	0%	0%	0%	0%	0%	0%	5	0%	0%	0%

Table 3. Least square Means (LSM) from General Linear Models (GLM) of Area Under the Curve (AUC) 1 months to 6 months after primo vaccination with multivalent BRD vaccines using serum Log 2 SN titres for BRSV and ELISA OD for BRSV. BPI3 and *Mh*

Serum test	Value	LSM	S.E	P-value	Lower CI	Higher CI	Sign diff
BRSV SN	Control	2.3	2.4	ref.	-2.5	7.0	b
BRSV SN	Bovalto	5.6	1.4	0.25	2.8	8.3	b
BRSV SN	Bovipast	12.9	1.5	<0.01	9.9	15.9	a
BRSV ELISA	Control	76.3	40.6	ref.	–3.2	155.8	b
BRSV ELISA	Bovalto	180.4	23.3	0.03	134.7	226.1	b
BRSV ELISA	Bovipast	322.9	40.6	<0.01	243.4	402.4	a
BPI3 ELISA	Control	220.5	50.6	ref.	121.2	319.7	c
BPI3 ELISA	Bovalto	387.4	29.3	<0.01	330.0	444.9	b
BPI3 ELISA	Bovipast	485.0	32.0	<0.01	422.3	547.7	a
***Mh* ELISA**	Control	293.1	64.6	ref.	166.5	419.8	b
***Mh* ELISA**	Bovalto	479.7	38.1	0.02	405.2	554.3	a
***Mh* ELISA**	Bovipast	521.7	41.7	<0.01	440.0	603.5	a

had elevated titres only during the first month after vaccination, and no antibodies detected thereafter, indicating that the titres might have been residual colostral antibodies. After the booster vaccination, 83 % of the Bovipast vaccinated calves seroconverted, whereas no calves in Bovalto or control group seroconverted.

The baseline serum titres for BRSV SN, BRSV ELISA, BPI3 ELISA and *Mh* ELISA (AUC-base) at the time of the first and second injection of the primo vaccination (Table 1) were not significantly different between groups, and there was no significant influence of age at vaccination (T0-age) on AUC-base (analysed separately in 4 separate GLM models, models not shown, can be obtained upon request).

The primo vaccination resulted in varied vaccination response in the groups in the period T0+1m to T0+6m (Table 1) for the various agents. Four separate GLMs evaluated the AUC-primo with AUC-base and T0-age as covariates (Table 3). BRSV Log2 SN AUC-primo was significantly higher in Bovipast vaccinated calves (12.9) compared to Bovalto vaccinated calves (5.6) and control calves (2.3). Similarly, BRSV ELISA AUC-primo was significantly higher in Bovipast vaccinated calves (322.9) compared to Bovalto vaccinated calves (180.4) and control calves (76.3). BPI3 ELISA AUC-primo was significantly higher in Bovipast vaccinated calves (485.0) compared to Bovalto vaccinated calves (387.4) and both were significantly higher than control calves (220.5). *Mh* ELISA AUC-primo was significantly higher in Bovipast vaccinated calves (521.7) and Bovalto vaccinated calves (479.7) compared to control calves (293.1).

The booster vaccination (AUC-booster) GLMs accounted for the titre at the booster vaccination (T-booster) (Table 4). BRSV Log2 SN AUC-booster was significantly

Table 4. Least square Means (LSM) from General Linear Models (GLM) of Area Under the Curve (AUC) 1 month after booster vaccination with multivalent BRD vaccines using serum Log 2 SN titres for BRSV and ELISA OD for BRSV. BPI3 and *Mh*a

Serum test	Value	LSM	S.E	P-value	Lower CI	Higher CI	Sign diff
BRSV SN	Control	0.8	0.4	ref.	-0.1	1.7	b
BRSV SN	Bovalto	1.2	0.3	0.40	0.6	1.8	b
BRSV SN	Bovipast	3.7	0.3	<0.01	3.2	4.3	a
BRSV ELISA	Control	23.4	5.4	ref.	12.9	33.9	b
BRSV ELISA	Bovalto	74.1	3.4	<0.01	67.5	80.7	a
BRSV ELISA	Bovipast	87.9	3.4	<0.01	81.3	94.5	a
BPI3 ELISA	Control	109.9	2.6	ref.	104.8	115.0	b
BPI3 ELISA	Bovalto	113.2	1.8	0.30	109.8	116.6	b
BPI3 ELISA	Bovipast	121.5	1.7	<0.01	118.2	124.8	a
***Mh* ELISA**	Control	74.1	4.7	ref.	64.9	83.4	b
***Mh* ELISA**	Bovalto	84.5	3.1	0.08	78.3	90.6	ab
***Mh* ELISA**	Bovipast	89.1	3.0	0.01	83.2	95.0	a

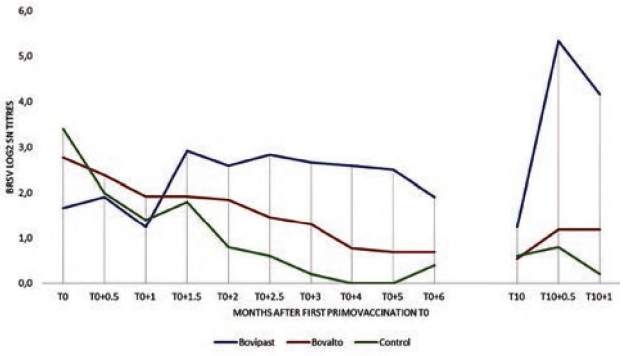

Fig. 1. Log 2 serum neutralisation titres against Bovine Respiratory Syncytial Virus in serum from calves vaccinated with multivalent bovine respiratory disease vaccines

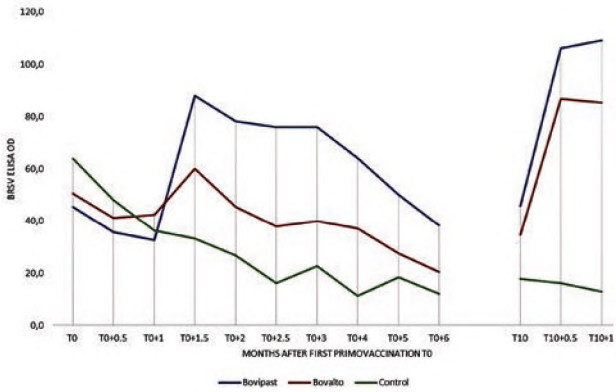

Fig. 2. Bovine Respiratory Syncytial Virus ELISA optic density measures in serum from calves vaccinated with multivalent bovine respiratory disease vaccines

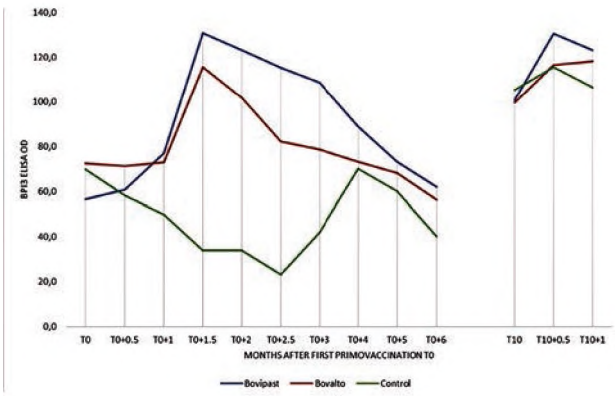

Fig. 3. Bovine Parainfluenza 3 ELISA optic density measures in serum from calves vaccinated with multivalent bovine respiratory disease vaccines

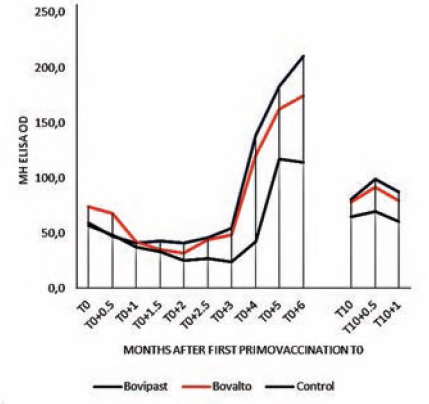

Fig. 4. *Mannheimia haemolytica* optic density measures in serum from calves vaccinated with multivalent bovine respiratory disease vaccines

higher in Bovipast vaccinated calves (3.7) compared to Bovalto vaccinated calves (1.2) and control calves (0.8). For BRSV ELISA AUC-booster, both Bovipast vaccinated calves (87.9) and Bovalto vaccinated calves (74.1) were significantly higher than control calves (23.4). BPI3 ELISA AUC-booster was significantly higher in Bovipast vaccinated calves (121.5) compared to Bovalto vaccinated calves (113.2) and control calves (109.9). *Mh* ELISA AUC-booster was significantly higher in Bovipast vaccinated calves (89.1) compared to control calves (74.1), whereas Bovalto vaccinated calves (84.5) were not significantly different from either group.

DISCUSSION

During the past few years, an increasing number of vaccines against BRD have been available on the European market and good availability of vaccines is a high priority in the European Union [10]. As most BRD vaccines have similar indications, it is not easy for veterinary practitioners and farmers to make evidence-based vaccine choices [8]. Meaningful comparisons between vaccines can only be done if the vaccines are tested under similar conditions. The Federation of Veterinarians in Europe has emphasized that vaccines are important to reduce antimicrobial use, but that there are lacking studies performed in dairy calves and lacking good field trials with natural exposure to pathogens [12]. In this study, 2 multivalent inactivated vaccines with similar pathogen spectra and adjuvants have been tested under field conditions as this is the most representative for the real situation.

The overall lack of respiratory disease in the calves in this study did not allow for evaluation of reduction in respiratory diseases between the vaccines and controls. Vaccines can be defined as generally effective if they prevent the disease which can only be proven by clinical and immunological efficacy studies. These studies are relatively expensive and are not fully in line with the Replacement, Reduction, Refinement principle of performing animal experiments [21]. However, serum antibody titres can be quite easily measured and the seroneutralisation assay used in this study for BRSV can to a certain extent indicate biological activity of the antibodies as this assay indicates that infectious agents are truly neutralized. Neutralizing antibodies are an integral part of the immune system and provide protection against many disease pathogens. Measurement of neutralizing antibodies provides an indication of the level of protection present in an animal and has been correlated with disease protection [14]. A four-fold serum antibody titre increase is often used as an indication of an appropriate vaccination response. However, this rule of thumb may seriously impact vaccination response in individuals with a high titre at the start of vaccination, and therefore a more flexible regression modelling approach may be preferable [1]. These SN tests may be preferable to evaluate immunity compared to simply quantitative tests such as the enzyme-linked immunosorbent assay (ELISA) that are not all specific for neutralizing antibodies or specific vaccines antigens. We were unable to find a reliable SN test for BPI3. Thus, in this study we used SN for BRSV, and the commercially available ELISA test K369 (BioX) for the 3 pathogens. Other facets of the immune system, particularly those related to cell-mediated immunity, may be of equal or greater importance in the control of viral pathogens. However, measuring cell-mediated immunity is expensive and cumbersome in field conditions with large numbers of animals and logistic limitations.

This field study indicated that BRSV SN response was different between the two vaccines and over time. Seroconversion indicated that higher percentage of Bovipast vaccinated calves seroconverted compared to the Bovalto vaccinated calves and the negative control calves. Only the Bovipast vaccinated calves show a high seroconversion rate after the booster vaccination. A reason for the failure of the seroconversion in the Bovalto group at the booster vaccination at 9–11 months of age may be the inhibition of priming by maternal antibodies in the passively immune calves [7]. Since seroconversion as measured at a single time may not well describe the temporal immunity of calves following vaccination and it is based upon an arbitrary cut point, this outcome was not further analysed statistically.

For further statistical analysis, the area under the curve from the second dose of primo vaccination until 6 months after the primo vaccination and one month after the booster vaccination was used. The AUC analysis indicated significantly higher BRSV antibody response in Bovipast vaccinated calves compared to Bovalto vaccinated calves and the negative control calves. The Bovalto vaccination was not significantly better than control. It may be claimed that there was a possible influence of colostral antibodies on the vaccination results as the BRSV titre at the first injection

was numerically higher in the Bovalto group compared to the Bovipast group. However, the statistical model shows that the AUC base titres were not significantly different between groups. Furthermore, the general linear model of AUC-primo was not significantly associated with AUC-base titres or age of calf at the primo vaccination.

Overall, this field study indicated elevated neutralising and humoral immunity to BRSV in Bovipast vaccinated calves after the primo and booster vaccinations, whereas Bovalto vaccinated calves had no significantly elevated titres compared to negative control calves. The humoral immunity to PBI3 was elevated after the primo vaccination in Bovipast and Bovalto vaccinated calves compared to negative control calves, however the humoral immunity decreased over the 6 months post-vaccination to levels of negative control calves and a significant elevation of humoral BPI3 immunity was detected only in Bovipast vaccinated calves after the booster vaccination. However, the BPI3 titres of the negative control calves also increased between 2.5 and 4 months, possibly indicating a natural infection. The humoral immunity against *Mh* was gradually increasing 2 months post-vaccination in Bovipast and Bovalto vaccinated calves most likely in response to a natural infection as a similar increase is also happening in the control group (Diagram 4). The absence of increasing titres for *Mh* after the primo vaccination may be explained by 2 reasons. The first hypothesis is a total or partly inhibition of the primo vaccination due to a high level of passive immunity received via colostrum. The second hypothesis is a difference in antigens between the ELISA BioX kits and the vaccines without cross detection. The latter hypothesis is most probably the most plausible as from previous testing (not published) and information from the developer of the BioX test kits (personal communication BioX), we know that ELISA BioX K369 and K139 are appropriate diagnostic kits to detect a natural infection but not always to detect responses to vaccination. The ELISA BioX kits are based on an LPS virulence factor of *Mh* which is most probably not present as antigen in the vaccines. This has been confirmed with an ELISA test that has been specifically developed to detect IRP antigens present in Bovipast. When analysing the samples of the Bovipast vaccinated animals with this specific ELISA in-house test, *Mh* primo vaccination and booster humoral significant responses could be confirmed (supplementary data can be obtained from corresponding author upon request). As for Bovipast, a specific ELISA test detecting antigens present in Bovalto could give a similar result. Based on these *Mh* results, it is very important to underline those commercial kits, BioX K369 and K139, are not able to detect if animals have been vaccinated and to estimate or compare vaccine responses. Additionally, these results indicate that it is important to know which antigens are in the vaccine as well as in the ELISA kit. Some *Mh* vaccines are based on capsular antigens, whereas some others on specific virulence factors (IRP, leukotoxins).

At the booster vaccination (AUC-booster), there was evidence of a memory response for BRSV, BPI3 but not for *Mh* for both vaccines. However, the lack of *Mh* increasing titres at booster vaccination may again be explained by the used ELISA kit as explained before (based on specific LPS *Mh* antigen). On the other hand, for the BRSV seroneutralisation response at booster, there was a significant lack of response in the Bovalto group. This can be explained by several hypotheses:

1. No cross neutralization response between vaccine strain and lab test strain. It may be interesting to repeat the seroneutralisation tests with several current strains for which BRSV and BPI3 cross protection is expected [23];
2. Denaturation of immunogenic antigens during the inactivation process of the vaccine strain [24];
3. Inhibition of priming humoral immunity and long cell-mediated memory in field condition by maternal antibodies;
4. Antigen mass and adjuvant quality and quantities are not appropriate to give a detectable response in the current conditions.

The antigen origin and mass in both vaccines are different. The 2 adjuvants in the vaccines (Quil A and Aluminium hydroxide) are the same, however the concentration and absolute volume per dose is different, e. g., Quil A adjuvant can activate both the cell mediated and the antibody mediated immune responses to a broad range of viral, bacterial, parasitic and tumour antigens [22]. One dose of Bovipast contains more Quil A than one dose of Bovalto, which may contribute to overcoming the inhibitory effects of maternally derived antibodies on the vaccine immune response.

There are limited studies documenting the efficacy of calf immunization in the presence of maternal antibody protection. It has been described that passive immunity might interfere with the induction of an active immune response after vaccination [2, 16]. However, some vaccines

are effective in young calves, even in the presence of maternal antibodies [19, 24]. One study evaluated virus excretion and seroconversion following calves with maternal BRSV immunity receiving single dose of either live vaccine, inactivated vaccine, or negative control [19]. It was noticed that the virus excretion was reduced, and seroconversion was highest in the calves having received the inactivated vaccine. Another study indicated that, despite the lack of BRSV seroconversion following the administration of an inactivated vaccine, the vaccination of calves with maternal antibody protection can results in lower virus shedding and clinical signs of disease [20]. A good response following Bovipast vaccination in the presence of maternally derived antibodies has been described where a single vaccination with Bovipast was able to break through the maternal antibody immunity, prime the cellular immune response and induce partial protection in young calves [24]. This study further confirms that Bovipast vaccination can create a neutralising immune response to BRSV in the presence of maternal antibodies.

No significant BRSV neutralizing response was observed in conventional calves vaccinated with Bovalto despite the presence of the maternal antibodies at vaccination. However, vaccination with Bovalto might still provide partial clinical protection as it might induce cellular immunity. The latter has not been measured in this study as explained before. It has been described that in presence of the maternal antibodies, intranasal vaccines or natural infections can induce cellular immunity without neutralising antibodies; reduce excretion and prime neutralizing response when a homologous or heterologous booster is done with an injectable vaccine or at new natural exposure [9, 11, 17]. A fairly new approach in comparative vaccinology is the heterologous prime-boost method of immunization, using different forms of an antigen, administered by different routes to broaden and extend immune responses [17]. Heterologous boost with adjuvanted inactivated parenteral BRSV vaccines may provide a better boosting response for mucosal primed calves with an intranasal attenuated vaccine [6, 11].

From this and other studies, it is clear that Bovipast primo vaccination in young calves could induce a long memory and regenerate quickly neutralizing antibodies when a booster is done: 9 to 11 months in this study, until 12 months in another study [19] or in case of natural exposure which often occurs in the field.

CONCLUSIONS

Seroneutralisation tests are able to evaluate a part of protective immunity in contrast to some ELISA kits that could detect or not specific neutralizing antibodies (false positive) or specific vaccine antigens. Under field conditions, it is hard to evaluate and compare immunity induced by vaccines based on diverse ELISA kits that are currently on the market. There is a need to develop more appropriate tests to measure vaccine immunity in the field.

In addition, significant differences were found in serological values after Bovilis® Bovipast RSP vs. Bovalto® Respi 3 vaccination, supporting the notion that despite containing antigens targeting the same pathogens, immunogenicity of vaccines can be very different.

ETHICAL CONSIDERATION

The study was carried out in compliance with the relevant animal welfare legislation. The authors declare no animal ethical approval was needed. This study was carried out on one commercial dairy farm, so on "private lands". Based on an accurate description of the study's objectives and its design, the farm owner gave permission to the herd veterinarian to conduct the study on their farm. Except for this informed consent, no other specific permission was required. No calves were sacrificed for the purposes of the study.

ACKNOWLEDGEMENTS

We would like to extend our gratitude and thanks to the herd veterinarian and the farm for their study participation.

DECLARATION OF INTEREST

Thibault Jozan and Geert Vertenten are employees of MSD Animal Health, the company that markets one of the vaccines that have been used in the study reported herein. Anna Catharina Berge is an independent consultant at Berge Veterinary Consulting BV, performing consultancy for MSD-AH. Camille Levesque is employed at Laboceae, where serum analysis was performed.

REFERENCES

1. **Beyer, W. E., Palache, A. M., Luchters, G., Nauta, J., Osterhaus, A. D., 2004:** Seroprotection rate, mean fold increase, seroconversion rate: which parameter adequately expresses seroresponse to influenza vaccination? *Virus Res.*, 103, 125—132. DOI: 10.1016/j.virusres.2004.02.024.
2. **Chamorro, M. F., Woolums, A., Walz, P. H., 2016:** Vaccination of calves against common respiratory viruses in the face of maternally derived antibodies (IFOMA). *Anim. Health Res. Rev.*, 17, 79—84. DOI: 10.2460/ajvr.77.1.88.
3. **Delabouglise, A., James, A., Valarcher, J. F., Hagglund, S., Raboisson, D., Rushton, J., 2017:** Linking disease epidemiology and livestock productivity: The case of bovine respiratory disease in France. *PLOS ONE*, 12, e0189090. DOI: 10.1371/journal.pone.0189090.
4. **Dunn, T. R., Ollivett, T. L., Renaud, D. L., Leslie, K. E., LeBlanc, S. J., Duffield, T. F., Kelton, D. F., 2018:** The effect of lung consolidation, as determined by ultrasonography, on first-lactation milk production in Holstein dairy calves. *J. Dairy Sci.*, 101, 5404—5410. DOI: 10.3168/jds.2017-13870.
5. **Edwards, S., 1990:** The diagnosis of bovine virus diarrhoea-mucosal disease in cattle. *Rev. Sci. Tech.*, 9, 115—130. DOI: 10.1016/j.cvfa.2010.03.005.
6. **Ellis, J. A., West, K. H., Waldner, C., Rhodes, C., 2005:** Efficacy of a saponin-adjuvanted inactivated respiratory syncytial virus vaccine in calves. *Can. Vet. J.*, 46, 155—162.
7. **Ellis, J., Gow, S., Bolton, M., Burdett, W., Nordstrom, S., 2014:** Inhibition of priming for bovine respiratory syncytial virus-specific protective immune responses following parenteral vaccination of passively immune calves. *Can. Vet. J.*, 55, 1180—1185.
8. **Ellis, J. A., 2017:** How efficacious are vaccines against bovine respiratory syncytial virus in cattle? *Vet. Microbiol.*, 206, 59—68. DOI: 10.1016/j.vetmic.2016.11.030.
9. **Ellis, J., Gow, S., Berenik, A., Lacoste, S., Erickson, N., 2018:** Comparative efficacy of modified-live and inactivated vaccines in boosting responses to bovine respiratory syncytial virus following neonatal mucosal priming of beef calves. *Can. Vet. J.*, 59, 1311—1319.
10. **European Medical Agency, 2018:** *Report on the Operation of the Steering Group for the Joint EMA/HMA Action Plan on Availability of Veterinary Vaccines*. https://www.ema.europa.eu/en/documents/report/report-operation-steering-group-joint-ema/hma-action-plan-availability-veterinary-vaccines-2016-2017_en.pdf.
11. **Erickson, N. E. N., Berenik, A., Lardner, H., Lacoste, S., Campbell, J., Gow, S., Waldner, C., Ellis, J., 2021:** Evaluation of bovine respiratory syncytial virus (BRSV) and bovine herpesvirus (BHV) specific antibody responses between heterologous and homologous prime-boost vaccinated western Canadian beef calves. *Can. Vet. J.*, 62, 37—44.
12. **Federation of Veterinarians of Europe, 2016:** *Antimicrobial Use in Food Animals*. https://www.ema.europa.eu/en/documents/report/annex-replies-efsa/ema-questions-use-antimicrobials-food-producing-animals-eu-possible-measures-reduce-antimicrobial_en.pdf.
13. **Griffin, D., 2014:** The monster we don't see: subclinical BRD in beef cattle. *Anim. Health Res. Rev.*, 15, 138—141. DOI: 10.1017/S1466252314000255.
14. **Grooms, D. L., Coe, P., 2002:** Neutralizing antibody responses in preconditioned calves following vaccination for respiratory viruses. *Vet. Ther.*, 3, 119—127.
15. **Heldens, J. G., Weststrate, M. W., Van den Hoven, R., 2002:** Area under the curve calculations as a tool to compare the efficacy of equine influenza vaccines—a retrospective analysis of three independent field trials. *J. Immunol. Methods*, 264, 11—17.
16. **Kimman, T. G., Westenbrink, F., Schreuder, B. E., Straver, P. J., 1987:** Local and systemic antibody response to bovine respiratory syncytial virus infection and reinfection in calves with and without maternal antibodies. *J. Clin. Microbiol.*, 25, 1097—1106. DOI: 10.1128/jcm.25.6.1097-1106.1987.
17. **Kimman, T. G., Westenbrink, F., Straver, P. J., 1989:** Priming for local and systemic antibody memory responses to bovine respiratory syncytial virus: effect of amount of virus, virus replication, route of administration and maternal antibodies. *Vet. Immunol. Immunopathol.*, 22,145—160. DOI: 10.1016/0165-2427(89)90057-3.
18. **Lu, S., 2009:** Heterologous prime-boost vaccination. *Curr. Opin. Immunol.*, 21, 346—351. DOI: 10.1016/j.coi.2009.05.016.
19. **Makoschey, B., Laar, P., 2006:** Evaluation of the anamnestic response of BRSV and PI3 component of Bovilis Bovipast RSP after a single booster at 3, 6 or 12 months post vaccination. In *24th World Buiatrics Congress, Nice*. https://www.cabdirect.org/cabdirect/abstract/20073078906.
20. **Makoschey, B., Berge, A. C., 2021:** Review on bovine respiratory syncytial virus and bovine parainfluenza—usual suspects in bovine respiratory disease—A narrative review. *BMC Vet. Res.*, 17, 261—279. DOI: 10.1186/s12917-021-02935-5.
21. **Mawhinney, I. C., Burrows, M. R., 2005:** Protection against bovine respiratory syncytial virus challenge following a single

dose of vaccine in young calves with maternal antibody. *Vet. Rec.*, 156, 139—143. DOI: 10.1136/vr.156.5.139.

22. **Patel, J. R., Didlick, S. A., 2004:** Evaluation of efficacy of an inactivated vaccine against bovine respiratory syncytial virus in calves with maternal antibodies. *Am. J. Vet. Res.*, 65, 417—421. DOI: 10.2460/ajvr.2004.65.417.
23. **Russell, W. M. S., Burch, R. L., 1959:** *The Principles of Humane Experimental Technique, Methuen, London.* https://caat.jhsph.edu/principles/the-principles-of-humane-experimental-technique.
24. **Steff, A. M., Monroe, J., Friedrich, K., Chandramouli, S., Nguyen, T. L., Tian, S., Vandepaer, S., Toussaint, J., Carfi, A., 2017:** Pre-fusion RSV F strongly boosts pre-fusion specific neutralizing responses in cattle pre-exposed to bovine RSV. *Nat. Commun.*, 8, 1085. DOI: 10.1038/s41467-017-01092-4.
25. **Sun, H. X., Xie, Y., Ye, Y. P. 2009:** ISCOMs and ISCOMATRIX. *Vaccine*, 27, 4388—4401. DOI: 10.1016/j.vaccine.2009.05.032.
26. **Valarcher, J. F., Schelcher, F., Bourhy, H., 2000:** Evolution of bovine respiratory syncytial virus. *J. Virol.*, 74, 10714—10728. DOI: 10.1128/jvi.74.22.10714-10728.2000.
27. **Van der Sluijs, M. T. W., Kuhn, E. M., Makoschey, B., 2010:** A single vaccination with an inactivated bovine respiratory syncytial virus vaccine primes the cellular immune response in calves with maternal antibody. *BMC Vet. Res.*, 6, 2. DOI: 10.1186/1746-6148-6-2.
28. **Windeyer, M. C., Leslie, K. E., Godden, S. M., Hodgins, D. C., Lissemore, K. D., LeBlanc, S. J., 2015:** Association of bovine respiratory disease or vaccination with serologic response in dairy heifer calves up to three months of age. *Am. J. Vet. Res.*, 76, 239—245. DOI: 10.2460/ajvr.76.3.239.

Received September 2, 2021

Accepted October 11, 2021